

*What is claimed is:*

1. A method for obtaining a three-dimensional representation of a light source distribution located inside a sample, the method comprising:
  - 5 providing surface light image data from light emitted from a surface of the sample originating from the light source distribution located inside the sample; and
  - reconstructing a three-dimensional representation of the light source distribution internal to the sample based on the surface light emission data.
- 10 2. The method of claim 1 further comprising obtaining a topographical surface representation of the sample.
3. The method of claim 2 further comprising dividing the topographical surface representation into a set of surface elements.
4. The method of claim 3 wherein each surface element is approximated as planar.
- 15 5. The method of claim 3 further comprising creating a set of volume elements within the sample.
6. The method of claim 5 wherein each volume element is modeled to contain a point light source at its center.
7. The method of claim 6 wherein the three-dimensional representation of the light
- 20 source distribution is approximated by a set of point light sources.
8. The method of claim 5 further comprising converting the surface light image data into photon density just inside the surface of the sample.
9. The method of claim 8 wherein there is a linear relation between the light source emission strength in a given volume element and the photon density just inside a surface
- 25 element.
10. The method of claim 5 further comprising defining a cost function and a set of constraints for obtaining a solution for the three-dimensional representation of the light source distribution.

11. The method of claim 10 wherein the cost function is related to a sum of source strengths for each point source in the sample, and the constraints include the following conditions: (i) that the source strengths be positive definite and (ii) that the resulting photon density at the object surface produced by the distribution of point sources be  
5 everywhere less than the measured surface photon density.
12. The method of claim 11 wherein obtaining the three-dimensional representation maximizes the cost function subject to the constraints.
13. The method of claim 10 wherein the cost function and constraints are described mathematically by a system of linear equations, and a solution for the three-dimensional  
10 representation of the source distribution is obtained using a SIMPLEX method.
14. The method of claim 10 further comprising including a weighting factor in the cost function that can be varied to produce a set of solutions for the three-dimensional representation of the source distribution.
15. The method of claim 10 further comprising varying the number of surface  
15 elements to produce a set of solutions for the three-dimensional representation of the source distribution.
16. The method of claim 5 further comprising varying one of a) the number of volume elements, and b) the configuration of volume elements, to produce a set of solutions for the three-dimensional representation of the source distribution.
- 20 17 The method of claim 5 further comprising optimizing the three-dimensional representation of the light source distribution by calculating the surface light emission for each solution and selecting a solution which minimizes a difference between a calculated and measured surface emission.
18. The method of claim 16 wherein the varying the number of volume elements and  
25 varying the configuration of volume elements both comprise adaptive meshing.
19. The method of claim 18 wherein the adaptive meshing increases the number of volume elements used to describe the three-dimensional representation of the light source.

20. The method of claim 19 wherein the adaptive meshing removes volume elements having zero light source strength.
21. The method of claim 5 wherein the transport of light within the sample from a given volume element to a given surface element is described by a Green's function.
- 5 22 The method of claim 21 wherein the Green's function is defined as a solution for light diffusion in a homogenous half space having a planar boundary perpendicular to the line connecting the volume element and the surface element.
23. The method of claim 21 wherein the sample interior is approximated to be inhomogeneous.
- 10 24. The method of claim 21 wherein the Green's function is defined in a look-up table.
25. The method of claim 21 wherein the Green's function is calculated using Monte Carlo simulations or Finite Element Modeling.
26. The method of claim 1 wherein the light source is comprised of bioluminescent  
15 or fluorescent emission.
27. The method of claim 1 further comprising applying a noise threshold to the surface light image data.
28. The method of claim 27 wherein the noise threshold is related to one of the peak intensity in the surface light image data and the dynamic range in the surface light image  
20 data.
29. The method of claim 28 wherein the noise threshold is related to the peak intensity in the surface light image data divided by dynamic range in the surface light image data.
30. The method of claim 27 wherein the surface representation is divided into a set  
25 of surface elements and all surface elements having surface emission below the noise threshold are removed.

31. The method of claim 1 wherein the sample is an animal and the light source emits light that passes through animal tissue.
32. The method of claim 31 wherein the animal tissue is approximated to be homogenous.
- 5 33. The method of claim 1 wherein the sample has a complex boundary.
34. The method of claim 1 further comprising producing multiple possible three-dimensional representations of the light source and the three-dimensional representation of the light source obtained is the representation that best fits the measured surface light image data.
- 10 35. The method of claim 1 further comprising placing the sample on a stage included in an imaging chamber coupled to a camera configured to capture an image of the sample on the stage.
36. The method of claim 34 further comprising:  
moving the stage to a first position in the imaging chamber; and  
15 capturing a first image set of the sample from the first position using the camera.
37. The method of claim 35 wherein the first image set is comprised of a luminescent image, a structured light image, and a photographic image.
38. The method of claim 34 further comprising:  
moving the stage to one or more other positions in the imaging chamber, wherein  
20 the other positions have different angles relative to a fixed datum associated with the camera than the first position; and  
capturing a additional image sets of the sample from the other positions using the camera.
39. The method of claim 38 wherein obtaining the surface representation comprises  
25 building a topographic representation of the sample based on structured light data included in one or more structured light images.
40. The method of claim 34 wherein the surface light image data is obtained at one or more different wavelengths.

41. An imaging system for obtaining a three-dimensional representation of a light source located inside a sample, the imaging system comprising:
- an imaging chamber having a set of walls enclosing an interior cavity, and
- 5 comprising:
- a camera mount configured to position a camera;
- a moveable stage apparatus including a transport mechanism and a stage configured to support the sample within the interior cavity, the stage being coupled with the transport mechanism for movement of the sample to one of a plurality of positions in
- 10 the interior cavity;
- a light transport mechanism for transmitting light emitted from a surface of the sample to the camera; and
- a processor designed or configured to provide surface light image data from light emitted from the surface of the sample originating from the light source
- 15 distribution located inside the sample to obtain a three-dimensional representation of the internal light source distribution based on the surface light image data.
42. The system of claim 41 wherein the processor is further configured to build a topographical surface representation of the sample based on structured light data received by the camera.
- 20 43. The system of claim 41 wherein the transport mechanism is capable of moving the stage in two dimensions.
44. The system of claim 41 wherein the light transport device rotates about a fixed axis.
45. The system of claim 41 further comprising a structured light source configured to
- 25 transmit structured light onto the sample.
46. The system of claim 45 wherein the structured light source transmits structured light onto a mirror before the structured light reaches the sample.
47. The system of claim 41, wherein the light source is comprised of bioluminescent or fluorescent emission.

48. A computer program product comprising a computer readable medium and program instructions provided via the computer readable medium, the program instructions comprising reconstruction instructions for obtaining a three-dimensional representation of a light source distribution located inside a sample, the reconstruction
- 5 instructions capable of providing surface light image data from light emitted from a surface of the sample originating from the light source distribution located inside the sample and obtaining a three-dimensional representation of the light source distribution based on the surface light image data.
49. The computer program product of claim 48 further comprising instructions for
- 10 displaying the three-dimensional representation of the light source distribution.
50. The computer program product of claim 48 further comprising instructions for mapping light image data received by a camera onto a surface representation of the sample.
51. The computer program product of claim 48 further comprising instructions for
- 15 dividing the surface representation into a set of surface elements.
52. The computer program product of claim 48 wherein the light source is comprised of bioluminescent or fluorescent emission.